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Exploring Gene Functions and Phage-Host Protein Interactions in Mycobacteriophage Island3

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Island3 is an I1 mycobacteriophage that infects Mycobacterium smegmatis mc²155. It has a total of 76 protein coding genes, but only 17 of these genes have functions assigned by bioinformatics. To discover the functions of the additional genes, we cloned 72 of Island3’s genes and are assaying each gene product for two functions when expressed in the host M. smegmatis: the ability to reduce growth of the host (cytotoxicity) and the ability to protect the host from infection by Island3 or another phage (defense). So far, we have assayed more than 60 of Island3’s genes and found 14 genes that exhibited cytotoxicity but none that exhibited definitive defense against phage infection. We are currently analyzing the remaining genes for cytotoxicity and defense. In addition, we are moving forward with bacterial two-hybrid assays on two of the genes that exhibited cytotoxicity, seeking to identify host proteins that interact with the cytotoxic phage gene products in an attempt to understand the mechanism of cytotoxicity.